CONTRIBUTIONS TO THE STUDY OF DEVELOPMENT OF THE WING-PATTERN IN LEPIDOPTERA

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Introduction

The mechanism of pattern determination in Lepidoptera wings has long been a favorite model for the study of gene action. Every study of developmental processes is concerned with the study of development of manifold structure, which finds its best object in the study of pattern, e.g. the pattern of a butterfly-wing. Goldschmidt tried the first successful analysis of the factors responsible for the development of this pattern and expressed it in his theory of different velocities of developmental processes. Although most parts of his analysis could be experimentally checked and extended by Kühn and his school, the decisive observations ("relief-stage") of Goldschmidt's work were either not found, misinterpreted, or doubted by several investigators. An additional and more extended investigation of those stages was therefore necessary and will be furnished in this paper.[1]

Previous Experiments on the Development of the Wing-pattern

Goldschmidt's Investigations

In his book "Physiologische Theorie der Vererbung" (1927), Goldschmidt contributed an extensive analysis of the pattern problem.

A study of the development of the individual wing furnished the most decisive point in this analysis. Goldschmidt (1920, 1923) observed that the later wing-pattern is already completely represented in the young pupal wing, long before pigmentation takes place. It can be made visible by detaching unpigmented pupal wings from the body and allowing them to dry. After a short time a relief was visible on the former homogeneous-looking wing.

Later white scales became erected and formed the raised regions and later dark scales collapsed and formed the depressed regions. The whole relief represented the later wing-pattern in every detail. He observed this "relief-stage" in *Platysamia cecropia* L., *Lymantria, Thais*

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and several other species. Goldschmidt assumed that in certain parts of the pattern the scales are still soft bags filled with blood, which collapse when dried out. In other districts, however, the scales are already chitinized and do not collapse when dried out. Because the later dark scales are consequently softer at this point of development than the later white scales, he concluded that later dark scales exhibit a slower development than later white scales. He arrived at the conclusion that the different velocities of development for the different parts of the wing were primarily responsible for the formation of wing-pattern.

According to his theory the different velocities of development start with the mosaic of the epidermis cells and their product, the scales which develop with different speed in different parts of the wing. A section through the young pupal wing in a certain stage would accordingly show us different parts of the wing in different stages of development.

At the same time different chemical substances will be present in the body, which if deposited in the scales will determine the color. These substances, like tyrosine, carotine etc., can be end-products of metabolic processes and a special production of them for the coloration-processes is not necessary. The deposition of these substances in the scales can be dependent on a certain colloidal stage of the chitin. If at a certain stage in the development tyrosine is present, it will only be deposited in those scales which represent a certain condition of the chitin at this moment, which means only a certain part of the pattern. Other parts of the pattern will react according to their stage of chitin-development and the pigmentation substances present. The development of a very complicated pattern can thus be explained by a very simple mechanism.

Goldschmidt (1920, 1927) then tries to trace these processes back in development and discusses the determination-points for these differences in developmental velocities ("sensitive period," determination-stream, Liesegang phenomenon).

The Investigations of Kühn and His Co-workers

Kühn and his co-workers tried to attack the same problems and furnished further experimental material. They worked with the flour-moth, which shows a pattern consisting of dark and light pattern-elements which are partly symmetrical (see Fig. 1). The various parts of the pattern do not only contain different amounts of pigment but the shape and size of the scales in various districts differ also. Therefore, the determination of the pattern is here not only a process of distributing different amounts of pigment but a morphogenetic process as well.

Feldotto (1933) determined the sensitive periods for this pattern and showed that heat-treatment at the pupal age of 12–72 hours was able to modify the development of the different parts of the pattern. Each system of the pattern has its particular time of maximal modification at different stages of development.

Kühn and von Engelhardt (1933) cauterized the young pupal wing and thus were able to show that a determination-stream spreads over the wing from 48–60 hours of pupal age.

A period of particularly distributed cell divisions can be considered as the first reaction to the process of determination. These cell divisions were first described by Köhler (1932). He reported that from 36–84 hours of pupal age a great number of cell-divisions are visible which have their maximal distribution in districts of subsequent dark pattern elements while subsequent light pattern districts show few cell-divisions. The distribution of cell-divisions therefore resembles the subsequent pattern of pigmentation and Köhler consequently called it mitosis-pattern. A mitosis-pattern seems to be the predecessor to the later wing-pattern.

Braun (1936) examined the mitosis-pattern more closely with special regard to its rôle in the development of the wing-pattern.

He showed that this mitosis-pattern is visible from 36–148 hours of pupal age. Subsequent dark pattern-districts always represent maxima of mitotic divisions, while the minima are in later light pattern-districts during this period. The mitosis-pattern spreads in a wave-like fashion over the wing from the proximal base to the distal margin. Two such mitosis waves are distinguishable during the period of mitotic division. The first one goes over the wing from 36–84 hours, and represents cell-divisions of hypodermis-cells and the first division of the scale-building cells. The second wave starts at 84 hours and ends at 148 hours and represents the second division of the scale-building cells. When the first proximal divisions occur we find no mitosis in distal parts of the wing, with the exception of the cell-divisions of the later marginal scales, which start developing earlier and continue at a faster rate throughout the whole wing-development. Furthermore, it could be shown that this mitosis-pattern is the first sign of a completed determination of the wing-pattern.

Then an attempt was made to investigate the rôle of this mitosis-pattern in the development of the wing-pattern. It could be shown that the districts of mitosis-maxima (presumptive dark districts) represent districts of greater intensity of cell-division. Throughout the stages of development following the mitosis-period, later dark districts show a greater number of cells per unit than later white districts. The size of cells in the presumptive dark districts is accordingly smaller. This observation could be checked on the adult wing also, where one finds more scale-building cells per unit in dark districts than in light districts. In addition it was found that all scales grow out simultaneously. From these observations it was concluded that a different intensity of cell-division was primarily responsible for the formation of the pattern. The principle of different velocities of development was discarded in favor of the principle of different intensity of cell-division as the primary factor in the development of a pattern. Köhler and Feldotto (1937) again tried to discard the existence of different developmental velocities for Lepidoptera in a recent paper. Their argument is based on their failure to find the scales of different districts in different stages of growth during experiments on scale-development. Furthermore, it had not been possible to observe the relief stage in *Ephestia*.

In response to these views expressed by the *Ephestia*-workers, Goldschmidt showed in recent publications (1938) that the observations made on *Ephestia* are in perfect harmony with his discoveries. The fact that we find more cell-divisions in certain districts during the mitosis-period means that the cells in these districts have not yet reached the end of their multiplication period. These districts are the later dark districts, they show a retarded development not only in the relief-stage but at this early stage as well. The cells in later white districts show less cell-divisions because they have reached the end of their multiplication period earlier and therefore differentiate faster than the later dark cells. (An investigation concerning the number of divisions which a cell undergoes in later dark or in later white districts would probably prove this explanation. Such an investigation is now under way.) The observation of the simultaneous growing out of scales does not interfere with this theory, because the different speed of differentiation of the scales in the different districts does not necessarily mean different growth. The process of growth might be nearly identical for all wing areas, while the different speed of development should be noticeable by differences in the histological structure of the scales, which would be hard to examine at this early stage.

To test these views it was necessary to undertake a more detailed investigation of the relief stage, where the different velocity of development for the different pattern districts was demonstrated so clearly. Furthermore, an attempt had to be made to find additional evidence for the different speed of development at stages previous to the relief stage. The relief-stage had to be found in *Ephestia*, where the mitosis-period had been found. Finally the mitosis-period had to be observed in other Lepidoptera, where the relief-stage had been demonstrated previously. The results of these investigations, which fully confirmed the validity of Goldschmidt's views, are described in the following section.

New Investigations on the Determination of the Wing-pattern

Material and Methods

Ephestia kühniella Zeller, *Platysamia cecropia* L. and *Papilio ajax* L. were the objects of the experiments to be described. *Ephestia* cultures were kept according to the method described by Kühn and Henke. *Cecropia* and *Papilio* pupae were obtained in the fall and stored in a refrigerator, from which they were put into an incubator at any time desired. They started development as soon as they were placed in a warmer temperature. All pupae developed at 25°. Fifty pupae of *Platysamia cecropia,* fifty pupae of *Papilio* and approximately one hundred and fifty pupae of *Ephestia* were used for these investigations.

The Appearance of the Relief Stage

As pointed out before, *Ephestia*-workers have never been able to find the stage in the pupal-development which is decisive for the theory of different developmental velocities, namely the relief stage. Therefore the first step was to hunt for this decisive stage in the development of the flour-moth. In order to become acquainted with the stage in question, the author repeated Goldschmidt's (1920) experiments on the *Cecropia* moth and *Papilio.* The observations confirmed Goldschmidt's results completely.

Platysamia cecropia L.—The wings were removed from the pupae at a time when no pigmentation had yet taken place, the best time being one to two days before pigmentation. They were then placed on a slide and allowed to dry. A relief appeared which showed subsequent white parts erect, subsequent dark parts collapsed. This stage is relatively short, approximately 3–5 per cent of the whole pupal stage, and was observed on ten *Cecropia* wings.

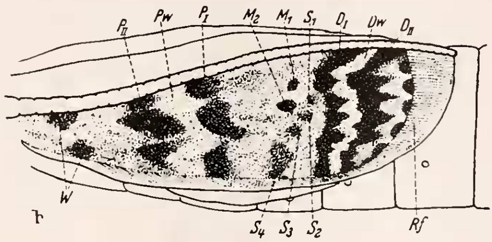
The relief of the *Cecropia*-wing is especially clear in the distal parts where the dark and yellow half-moon is located on the pigmented wing. (For excellent photos of this stage see Goldschmidt's 1920 paper.)

Papilio ajax L.—The same stage was easily found in *Papilio ajax.* Here too the relief-stage takes 3–5 per cent of the complete time of pupal development. It is very clear all over the wing, corresponding to the mature wing-pattern, and appears approximately twenty minutes after the wing has been put on a slide for drying. The subsequent light parts of the wing are indicated by clearly erected parts, the subsequent dark pigmented districts of the wing are all collapsed. This stage could be observed in fourteen wings which were removed from the pupae one to two days before pigmentation started. The different unpigmented pattern districts of the wings were visible even without drying. If the light was shone in a certain way on the still attached and living wing, the districts of subsequent dark pigmentation and light pigmentation were clearly distinguishable by what appeared to be a difference of consistency of the areas in question. This difference can be demonstrated even more clearly, if one puts the wing into water. Again the districts of subsequent different pigmentation can be easily distinguished on the completely unpigmented wing. The action of two time factors for this period could be observed in *Papilio*. (1) At the moment pigmentation started, the relief disappeared in pigmented parts, while unpigmented parts showed the relief distinctly. (2) It is known that the hind wing always shows a faster development than the fore wing (e.g. pigmentation). Consequently the relief was already present on the hind wing, while all scales were still soft on the fore wing. The relief of the hind wing disappeared earlier because pigmentation set in earlier. (Pictures of the relief stage in species closely related to *Papilio* can be found in Goldschmidt's 1923 paper.)

Ephestia k.—The corresponding stage was found without much difficulty in *Ephestia* at eight to nine days of pupal age. That it had never been noticed before is surprising. The wing of the pupa was easily detached after the surrounding chitin was broken and carefully removed. Then the wing was placed on a slide and allowed to dry. After about fifteen minutes the two later dark bands appeared clearly as collapsed parts on the dried wing (Figs. 1 and 2). The action of an additional time-factor could be observed on these wings. They showed that the relief-stage proceeds like many other processes in the development of the flour-moth (mitosis-pattern, pigmentation) in a wave-like fashion from the proximal base to the distal margin of the wing. In early stages the relief of the proximal band only was visible, while in distal parts all scales were collapsed (Fig. 2 *a*). A little later the whole wing showed the relief, scales both of the proximal and distal bands being collapsed (Fig. 2 *b*). The marginal scales were an exception. They always exhibited a faster development, first noticeable during the mitosis period, and they were already erected in distal " white " parts in the early relief-stage. As in *Papilio,* the districts of subsequent different pigmentation were visible on the undried wing if the light was reflected in a certain way, as well as when the wing was put into water. These observations were based on a study of approximately sixty pupae.

The Chitinization of Scales during Pupal-development as Proof of the Different Velocity of Development

Sections.—The fact that no regional differences in the speed of outgrowing scales could be detected has been mentioned before (Köhler, 1932; Braun, 1936; Köhler and Feldotto, 1937). It was necessary to make sections of the wings in the relief stage to see if erect scales show a morphological difference at this stage. Such sections were made from



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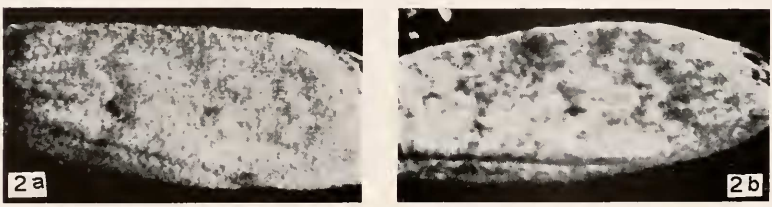

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FIG. 1. Pigmented pupal wing of *Ephestia kühniella*. P_I, P_{II} proximal dark bands, D_I, D_{II} distal dark bands, M_I, M_{II} middle-spots.

FIG. 2*a*–*b*. Relief-stage of the pupal wing of *Ephestia kühniella*. *a*, early relief-stage showing the proximal band clearly, *b*, later relief-stage showing proximal band, distal band and five marginal spots. × 9.

FIG. 3. *Papilio ajax*. × ⅔.

eight *Cecropia* wings (through the district of the "half-moon"), from twelve *Ephestia* wings, and six *Papilio* wings, but no morphological differences in the stage of development of collapsed or erect scales of different forms were noticed. In many Lepidoptera white and dark areas are composed of scales of different forms, correspondingly later dark parts showed larger scales on the *Cecropia* wing than later white parts.

Consequently the different speed of differentiation of the scales in the different pattern districts does not manifest itself by different stages of growth. Growth seems to be the same for all parts of the wing. The difference therefore has to be sought in different velocities of the chitinization process of the later white and dark scales at this stage as suggested by the soft condition of later dark areas.

Chemical Tests.—In fact, such a difference could be detected by chemical reactions. There is not much known about the chemistry of chitin, but P. Schulze (1922) described a differential reaction for hard and soft chitin. The object is first saturated with iodine for a short time, blotted with filter paper, and finally covered with a solution of $ZnCl_2$. The reaction will show very soon but disappears after some time. Hard chitin will show a violet-brown color, soft chitin, a light brown. Ten unpigmented *Cecropia* wings were treated accordingly and showed a distinct reaction. Subsequent white parts appeared dark; subsequent dark parts appeared light. This experiment exhibits clearly that the different parts are found in different stages of chitinization. The same experiment was repeated with six pupae of *Papilio* and twenty pupae of *Ephestia* and always resulted in a clear pattern negative. Even the sections which showed no morphological difference reacted distinctly to this chitin test. The chitin reaction worked during the whole relief stage. It would have been desirable to check this test by making the same experiment with an adult pigmented wing. In this case all scales should show the violet-brown reaction. However, it was not possible to extract the pigment from an adult wing, which would have been necessary in order to obtain a bleached wing on which the reaction would be visible. Several other chitin reactions were tested, but none of them gave as clear a result as did the iodine-zinc reaction.

Test with Polarized Light.—After it had been proven that the different speed of development actually can be shown to be the different velocity of chitin hardening, an experiment to test the refraction of the differently chitinized scales was tried. Adult scales are doubly refracting, and it was hoped that one could find a stage where one kind of scales was singly refracting. Scales from different regions were investigated under crossed Nicols, but no clear results were obtained. It was mentioned before that the scales from different regions have different forms, and this difference may add to different refraction effects.

Tyrosine Reactions.—The next experiment was an attempt to produce premature pigmentation artificially in order to watch the reaction of the different regions. Studies on the chemistry of melanin formation by several authors have shown that two components are necessary for pigmentation: a chromogen and an oxydase. Oxydase (e.g. tyrosinase) is always present in the blood of Lepidoptera as may be easily shown, while the chromogen is supposed to enter the wing at a definite stage of development (the time of pigmentation). Oxydase and oxygen together produce a melanin pigment. If the blood of Lepidoptera contains tyrosinase it should be possible to produce a dark pigment on the yet unpigmented wing by providing the necessary chromogen, e.g. by soaking the unpigmented wing in a solution of tyrosine.

(1) *Papilio ajax.*—An unpigmented pupal wing of *Papilio* at the time of the relief stage was put in a saturated solution of tyrosine. At the stage in question the pupal wing is always folded in a definite manner, which allows for the subsequent expansion of the wing after hatching. The wing is compressed in length and width to one-third of its actual size. This results in a wave-like appearance of the epithelium, with the scale-bearing epithelium as the crest and the epithelium without scales as the trough. (These folds do not interfere with the observation of the relief-stage on the pupal wing.) After immersion in the solution for four hours at 30°, the wing exhibited the following characteristics: it was completely stretched like the wing of the imago and showed the complete pattern of an adult pigmented wing; subsequent dark parts were dark, subsequent light parts were white (unpigmented) (Figs. 3 and 4). Microscopical examination of wings which had been treated with tyrosine revealed that the pigment is actually deposited in the scales. It is, however, not as black as the normally deposited pigment in the wing of the hatched butterfly. This experiment shows that a certain viscosity of the chitin is necessary for the deposition of a pigment in colloidal solution. After a scale has reached a certain point of hardening it cannot deposit any more pigment. The tyrosine can react with the tyrosinase in the still soft scales of subsequent dark parts, but no reaction can take place in the hardened scales of subsequent light parts. The tyrosine experiment demonstrates clearly how the different speed of hardening in different regions leads to the pattern of pigmentation.

Pupal wings of different age were treated accordingly to investigate the length of the stage during which a tyrosine reaction is possible. Very young and soft pupal wings, those on which the scales have just started to grow, show after treatment with a tyrosine solution a light gray color all over the wing with very faded contrasts of dark and light areas. The veins, however, and the marginal scales show a dark black pigment (Fig. 5). A control wing, dried at the same stage, shows all scales collapsed. In a later stage a dried-out wing will still show all scales collapsed, but if it is treated with tyrosine, a pale pattern will become visible, and subsequent dark parts will show black reaction, while subsequent light parts will not show any reaction with tyrosine (Fig. 6). With the help of the tyrosine reaction we thus can trace back the differential velocity of chitinization to a very early point in the scale de-

Fig. 4*a–b*. Pupal wings of *Papilio ajax* after treatment with tyrosine ca. 1 day before pigmentation begins to set in. *a*, fore wing and hind wing × 1½, *b*, part of the fore wing × 2½.

Fig. 5. Pupal wing of *Papilio ajax* after treatment with tyrosine ca. 2 days before pigmentation begins to set in. × 2½.

Fig. 6. Pupal wing of *Papilio ajax* after treatment with tyrosine ca. 3 days before pigmentation begins to set in. × 2½.

velopment. Long before the relief stage we are able to produce the later pattern, which means that before we can see the roughly morphological difference of the chitinization in the relief stage, we are able to demonstrate it by chemical means. The chitin of subsequent white scales either hardens earlier and does not allow the tyrosine to penetrate, or at these moments the white scales do not contain sufficient blood which, as carrier of the oxydase, is necessary for the reaction with the tyrosine. The first explanation seems more plausible. All the wings which showed the tyrosine reaction still have to be examined microscopically more carefully to complete our knowledge of the details of this process. But the fact remains that from a very early stage, the different velocities of the different districts can be shown long before we can observe collapsed or erected scales after exposure of the wing to air. The tyrosine reaction can be performed until normal pigmentation sets in on the wing and always gives a clear positive of the pattern. Eighteen wings of *Papilio* were used for these observations and *Papilio ajax* has proved to be an excellent object for these studies.

(2) *Ephestia k.*—The tyrosine reaction was tested on thirty flour-moth wings but did not show the bands distinctly, probably because the parts of the pattern of the *Ephestia* wing are not clear and limited enough.

It has been mentioned above that the pupal wings which are folded when removed from the pupae will always unfold after they have been placed in tyrosine. No other solution tested produced this phenomenon. Pupal wings from several species, among others pupal wings of *Drosophila*, were put in a tyrosine solution, and they always unfolded after one or two hours. This fact might be of great help for other embryological work in insects.

Dissolution of the Scales.—The next test was based on the following consideration. The scales show a distinctly different chitinization at the relief stage. It is easy to remove scales from the collapsed or erect parts. If the scales from both parts could be dissolved, the harder scales should need a longer time for dissolving. Mature scales on the wing of a hatched butterfly should show the same degree of chitinization in all parts, and white and dark scales should need the same time for dissolution. Such an experiment could furnish another proof that the different chitinization during development is really an expression of the differential speed of some developmental processes. All our experiments have a greater meaning if it can be proved that all scales are chitinized equally on the adult wing.

The experiments proved that this idea was right.

Papilio ajax scales from subsequent light and dark parts were dissolved in concentrated sulfuric acid. Subsequent dark scales start to dissolve as soon as touched by the acid. Subsequent white scales first show air bubbles when put into H_2SO_4, and then start to dissolve slowly (ca. 12 minutes). If the same experiment is repeated with scales from a mature pigmented wing, the white scales will show the same reaction as they did during the relief stage. This means they were already mature at this age. Dark pigmented scales, however, now show a longer resistance to the acid and start to dissolve only after hours of treatment. The prolonged time of the dark scales as compared with that of the white scales is probably due to the pigment deposition in the dark scales.

Scales from the same wing which were utilized for the observation of the relief-stage were used for this experiment. The same results were obtained in fifteen successive tests.

The Mitosis-pattern in Cecropia and Papilio ajax

In order to construct a general picture of the elements important for the pattern formation it was finally necessary to show that all the stages discussed (mitosis-pattern, different chitinization, relief-stage) are of general occurrence. Only the general presence of all stages, in particular relief-stage and mitosis-period, allows us to correlate them. The mitosis-pattern of *Ephestia* has been clearly demonstrated and closely investigated previously (Braun, 1936). The same kind of mitosis-pattern has been found now in ten wings of young *Cecropia* pupae, and follows the same principles as in *Ephestia*.

(The youngest *Papilio* pupae which we had in our laboratory were already too old to show the period of cell division.)

These observations confirmed that the mitosis-pattern, as well as the relief stage, is a general stage in the development of the wings of the Lepidoptera.

Discussion

The new evidence gained by these experiments confirms Goldschmidt's views completely. The tyrosine reaction, the relief stage and its chitin reactions, and finally the test of dissolving scales, leave no doubt that we are dealing with different velocities of development for the different parts of the pattern. This difference turned out to be a difference in the hardening or maturing of chitin. Under these circumstances it is only logical to interpret the mitosis-pattern in these terms too. We have seen that all the stages of development for different parts of the pattern are generally present in such different species of Lepidoptera as *Ephestia, Papilio ajax* and the *Cecropia* moth. *Ephestia*-workers neglected Goldschmidt's views on the basis of not finding different districts of the pattern in different stages of development, but they only considered growth, which does not seem to be an exact indicator for the different velocities. The indicator for these velocities seems to be of a purely histological nature. We have been able to demonstrate such an indicator in the process of chitinization of the scales. In interpreting the mitosis-pattern in the same terms as the subsequent stages of development we are aided by the similarity of time factors in both stages. The mitosis-period and the relief stage proceed wave-like proximally to distally over the wing. In both periods we find the marginal scales advanced in development as compared with all the other scales.

Based on former investigations and our new experiments we can describe the development of the pattern in Lepidoptera as follows:

In the very young pupal wing, districts of different visible as well as physico-chemical structure are present, partly produced by the localization of tracheas, veins and different surface conditions, perhaps also by processes in which a Liesegang phenomenon is involved. At a certain time in development a determination stream (or more than one) spreads over the wing and is distributed pattern-like according to the chemico-physical conditions present in the substrate and dependent upon the points of origin and directions of the stream or streams. In the case of *Ephestia* it can be shown that the subsequent dark districts are determined in the areas where the determination-stream stops. At any time before the occurrence of the determination-stream the subsequent pattern can be altered by extreme environmental conditions like heat treatment. Different parts of the pattern can be altered at definite limited periods, the " sensitive periods." Different phenotypes or genotypes are produced by the change of time-action of the determination-stream. The determination-stream determines districts with different speeds of differentiation. These different velocities of development are first visible in the mitosis-period which follows the period of the determination-stream. During this mitosis-period more cell-divisions are visible in later dark districts than in subsequent white districts, thus forming a mitosis-pattern, equal to the later pattern of pigmentation. The cells of the subsequent dark parts, which show a more intensive division process, have not yet reached the end of their multiplication period, in contrast to the subsequent white parts which enter their period of differentiation first. The mitosis-pattern spreads over the wing in the form of a mitosis-wave from the proximal base to the distal margin of the wing. Two such mitosis-waves can be observed. The first one is composed of cell-divisions of the hypodermis-cells and the first divisions of the scale-building cells. The second one is composed of the second cell-divisions of the scale-building cells. The different velocity of development for the different parts of the pattern can be observed again some time before pigmentation sets in. It can be made visible by the tyrosine reaction, the relief stage and its chitin reactions, and by the test of dissolving scales. These tests show that the subsequent dark parts, which have already been retarded during the mitosis-period, develop slower up to the time of pigmentation. The velocity of hardening of the chitin is different for the different parts of the pattern, and this difference finally leads to the pattern of pigmentation. Since a pigment is deposited in the chitin in colloidal solution, it can only be deposited as long as a scale has not reached a certain point of hardening. At a certain time of development pigment is present in the body and the subsequent dark parts, being still soft at this time, will deposit pigment; the subsequent white scales on account of their faster development, which has led to a harder chitinization, cannot deposit any pigment at this point. In this way the different velocities of differentiation of different areas of the wing throughout the pupal development starting from the time of the determination stream will lead to the pattern of pigmentation.

Summary

1. Goldschmidt's observations and ideas on the problem of different velocities of development for different parts of a pattern are described. The experiments of Kühn and his school and their negative views in regard to the idea of different velocities of developmental processes are summarized.

2. New observations confirm the existence of the " relief-stage " in the pupal wing of *Platysamia cecropia* and *Papilio ajax,* as well as in *Ephestia k.,* where it has not been previously observed.

3. It was observed that the relief-stage proceeds over the pupal wing from the proximal base to the distal margin in a wave-like fashion as is the case with the mitosis-pattern. The relief stage and the mitosis-pattern appear on the hind wing earlier than on the front wing.

4. No difference in growth of the scales was observed in sections through the wing during the relief-stage.

5. With the help of a chitin-reaction it could be shown that during the relief-stage the subsequent light parts of the wing were more chitinized than the subsequent dark parts.

6. A test with polarized light during the relief-stage gave no clear results.

7. With the help of artificial pigmentation (tyrosine-reaction) a complete pattern can be produced on the still unpigmented wing long before the relief-stage could be observed.

8. This tyrosine-reaction shows that a certain condition of the chitin is necessary to deposit pigment in the scales. Subsequent light parts of the wing are more chitinized at the time of pigmentation than subsequent dark parts and therefore cannot deposit any pigment. This experiment proves that the different velocities of development of the different parts of the pattern lead to the pattern of pigmentation.

9. By dissolving scales from subsequent dark and light districts in H_2SO_4 during the relief-stage and dark and light scales on the mature

wing, it could be actually shown that the scales are differently chitinized at the time of pigmentation and equally chitinized on the mature wing.

10. The similarity of processes during the mitosis-period and the relief stage and their general appearance in different species of Lepidoptera allow us to correlate them. Both stages express the different velocities of developmental processes for different parts of the wing, which finally produce the pattern of pigmentation.

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